

**HIGH TEMPERATURE RESPONSES IN TWO EXOTIC
LEAFCUTTING BEE SPECIES: *MEGACHILE APICALIS*
AND *M. ROTUNDATA*
(HYMENOPTERA: MEGACHILIDAE)**

JOHN F. BARTHELL¹, JOHN M. HRANITZ^{1,3}, ROBBIN W. THORP², AND
MARIA K. SHUE^{1,4}

¹Department of Biology, University of Central Oklahoma,
Edmond, Oklahoma 73034

²Department of Entomology, University of California, Davis, California 95616

³Current address: Department of Biological & Allied Health Sciences,
Bloomsburg University of Pennsylvania, Bloomsburg, Pennsylvania 17815

⁴Current address: Children's Hospital of Oklahoma,
Oklahoma City, Oklahoma 73104

Abstract.—Two exotic leafcutting bee species, *Megachile apicalis* and *M. rotundata*, have established feral populations in the Central Valley of California. Females of both species nest in exposed, oak savannah habitats where in the summer months ambient temperatures regularly exceed 40.0° C. Sympatric native bee species in the Central Valley of California are found most abundantly in insulated riparian zones where relatively cool temperatures and high humidities prevail. We examined physiological responses of *M. apicalis* and *M. rotundata* to high temperatures in the laboratory. Prepupal stage bees (in cocoons) were subjected to nine, three-hour temperature treatments of approximately 2.5° increments from 35.0° to 55.0° C. *Megachile apicalis* had significantly lower levels of the stress protein “heat-shock 70” (hsp70) than *M. rotundata* at all but the three highest temperature treatments. In a separate trial, hsp70 levels did not differ between species after four days of acclimation at 25.0° C but diverged significantly after a three-hour heat-shock treatment comparable to the ones described above. Both species survived temperatures up to 47.5° C, but began to differentiate around 50.0° C with *M. apicalis* demonstrating higher survivorship at or above that temperature. These findings indicate both species are highly thermotolerant, but agree with field data indicating that *M. apicalis* is more tolerant of high temperatures than *M. rotundata*.

Key Words.—Insecta, heat-shock or stress protein, invasive species, survivorship, thermotolerance.

Three exotic leafcutting bee species in the subgenus *Eutricharaea* have established feral populations in the U.S.A. during the 1900s: *Megachile apicalis* Spinola, *M. rotundata* (Fabricius) and *M. concinna* Smith (Cane 2002). These Old World species now appear to be establishing different geographic ranges within the western U.S.A. *Megachile concinna* is a southerly-distributed species in California that extends into the southwestern states of Arizona and Oklahoma. *Megachile rotundata*, the alfalfa leafcutting bee, has extended its range from northern California into the Pacific Northwest (U.S.A.) while *M. apicalis* reaches its highest densities within the Central Valley of California but also extends into southern California and the northern states of Oregon and Washington (Thorp et al. 1992, Thorp 1996, Frankie et al. 1998, Thorp et al. 2000, Barthell et al. 2002). Both species are multivoltine in California, flying from May into October with *M. rotundata* initiating nesting earlier in the season but with *M. apicalis* dominating in late summer and autumn (Stephen 1987, Thorp et al. 1992).

In an earlier study, both *M. apicalis* and *M. rotundata* were found to nest in

open, exposed habitats such as oak savannah, a pattern that contrasts with the nesting densities of sympatric native species which are restricted to riparian and marsh habitats (Barthell et al. 1998). Temperature and humidity patterns differ markedly between these habitats, with the more insulated marsh and riparian habitats having lower maximum and higher minimum biweekly temperatures (Barthell 1992). In the Central Valley of California the highly invasive *M. apicalis* nests in exposed microhabitats with ambient temperatures that can exceed 50.0° C. Nonetheless, over 60.0% of its offspring survive these conditions (Barthell et al. 1998). The tendency for *M. apicalis* to nest in and survive such thermal extremes, coupled with its more southerly distribution in California, suggest that it has higher thermotolerance than *M. rotundata*.

We examined responses to high temperature by *M. apicalis* and *M. rotundata* to test the hypothesis that these species have differing thermotolerances in accordance with their current distributions in California. We did so through measurement of stress protein levels (in the heat-shock 70 family of proteins) and survivorship. Heat-shock proteins are a well-documented indicator of stress in other organisms and heat-shock 70 proteins show a specific role in response to heat-induced stress (Feder & Hofmann 1999). No previous studies have measured either of these factors comparatively among solitary bees. Although both species appear to resist high temperatures well, we predicted that *M. apicalis* would have a more heat-tolerant thermal profile than *M. rotundata*.

MATERIALS AND METHODS

Origin of Live Material.—*Megachile rotundata* specimens were obtained as prepupae within brood cells from International Pollination Systems (IPS) in Manitoba, Canada. *Megachile apicalis* prepupae originated from field collections made at the Cosumnes River Preserve in the Central Valley of California. Sampling units consisted of 0.65 cm diameter $\times$ 15.5 cm length straws inserted into pre-drilled holes in 9.0 $\times$ 9.0 $\times$ 16.0 cm wooden blocks. These nests were set out in the field on 17 July and picked up on 10 August, 2000, to obtain second generation larvae that would diapause later in the season. Except during transport, brood cells of both species were kept in cold storage at their respective origin points but were refrigerated together under the same conditions (ca. 0.0°–15.0° C) for ca. three weeks (beginning on 14 February, 2001) before using the experimental protocol described below.

A second set of cells was collected the following year (2001) on 9 December from wooden trap-nest sampling units that were set out in the field on 9 August. All extracted straws (taped to 21.5 $\times$ 28.5 cm sheets of paper) collected from Cosumnes Preserve with brood cells of *M. apicalis* were x-rayed using a Hewlett-Packard 43805N Faxitron machine. Nests were exposed (3mA, 30 kVp) on Kodak XTL-2 film, allowing detection and removal of most parasitized cells used during Trials 1 and 2 described below. *Megachile apicalis* cells harvested from 0.50 cm diameter trap-nests in 2001 were used in Trial 3 (below) as well as new *M. rotundata* cells harvested during 2001 by IPS. Brood cells of both species were stored together for ca. 11 weeks (beginning on 8 January, 2002) at 5.0° C before the trial.

Trial 1: Tolerance to High Temperatures.—Brood cells (containing prepupae) of both *M. apicalis* and *M. rotundata* were removed from refrigeration and placed

at room temperature (ca. 20.0–25.0° C) on 8 March 2001. Cells were separated by species and treatment and then stored in petri dishes until heat-shock treatments began on 12 March. Approximately 110 brood cells were placed into each dish and color-coded with small, light streaks of Liquid Paper® (The Gillette Company, Boston, Massachusetts) to facilitate species identification.

Brood cells of both species were randomly mixed onto a single styrofoam plate for each temperature treatment and placed into a Little Giant® 9200 still air incubator (Miller Mfg., St. Paul, Minnesota) for three hours (11:00–14:00). Nine simultaneous temperature treatments were used, including approximately 2.5° increments from 35.0° to 55.0° C. (The exception was the 50.0° C treatment which actually received a 49.0° C exposure.) All plates with brood cells were simultaneously removed and allowed to cool at room temperature for 60 min after the three-hour trial. Twenty-five cells were randomly removed from each plate and immediately frozen at –80.0° C. The remaining cells were sorted by treatment and species into plastic petri dishes and incubated at 30.0° C. We report survivorship as well as both qualitative (SDS-PAGE) and quantitative (ELISA) measurements of stress protein levels (as described below) for this trial. Survivorship and protein levels were analyzed for significant differences between species using a Wilcoxon signed-rank test and a two-factor ANOVA, respectively.

Trial 2: Survivorship at 50.0° C.—To better differentiate the survivorship between species (the 49.0° C described above showed only incipient mortality), we subsequently exposed additional prepupae of *M. apicalis* and *M. rotundata* to 50.0° C (100 brood cells per species) on 7 May 2001. The same experimental conditions as described above were used and the experiment began at approximately the same time of day. To avoid parasitoid contamination (see below), the treated brood cells were placed in petri dishes which had been coated (inside bottom and lid) with a sticky layer of Tanglefoot® (Grand Rapids, Michigan). The brood cells were spaced equally across the bottom of each dish and then allowed to emerge in the 30.0° C incubator as before. We censused survivorship in this trial without examining hsp70 levels.

Trial 3: Stress Proteins in Acclimated vs Heat-shocked Larvae.—We observed unexpectedly high levels of hsp70 during Trial 1, even at all lower temperatures (including 35.0° C). To determine whether baseline levels of hsp70 in *M. apicalis* and *M. rotundata* were equivalent before heat-shock, we exposed groups of 25 prepupae of each species to simultaneous three-hour treatments of 25.0° and 35.0° C after a four-day acclimation period at 25.0° C. The work was conducted a year after Trial 1 (25 March, 2002) with prepupae collected during 2001 (see Materials and Methods). Otherwise, the same protocol as Trial 1 (except cold-storage temperature) was used to compare treatment and species differences in stress protein levels. A *t*-test was used to test for significant differences in protein levels between species.

Censusing Survivorship (Trials 1 & 2).—All brood cells (in their respective petri dishes) were removed for examination (including unemerged cocoons) four weeks after Trial 1. For Trial 2, cells were allowed to incubate approximately six weeks at 30.0° C before being removed from the incubator. These incubation periods meet or exceed those recommended for emergence of *M. rotundata* from Canada at 30.0° C (Peterson et al. 1992). After incubation, each cocoon/specimen

was scored as being in one of four categories as indicated below (samples were kept refrigerated between successive examination sessions to slow decay).

Parasitoids encountered during the study included a *Pteromalus* sp. that originated from *M. rotundata* cells. Among *M. apicalis* brood cells, the parasitoids included a wasp species in the family Leucospidae and numerous individuals of a *Melittobia* species. Some *Melittobia* (apparently not detected earlier by x-rays) parasitized brood cells of both bee species in all petri dishes (they were small enough to disperse under the petri dish lids to all other dishes). This required assessing survivorship according to four categories, only three of which were required for Trial 2 since no parasitoids emerged from those study specimens. The categories include the following: 1) *Emergent adults* (scored as surviving) that had chewed through and departed from their cocoons; 2) *Parasitized pre-adults* (scored as surviving) that included any unemerged bees in either the prepupal or pupal stage with feeding damage by and development of parasitoid larvae; 3) *Non-parasitized prepupae* (scored as dead) that included prepupae without obvious feeding damage by parasitoids. Any parasitoid larvae associated with these prepupae were small and morbid in appearance, indicating they had arrived at the prepupae after it died from heat exposure; 4) *Previously dead larvae* (excluded from analyses) that included unemerged larvae without parasitoid damage but that had obviously been dead prior to the heat treatment as evidenced by their hardened and/or darkened state (some of these had been killed by chalkbrood infection).

Although larval mortality appears to differ between male and female *M. rotundata* (e.g., Undurraga & Stephen 1980), we could not consistently record this variable because of parasitoid damage to specimens (see categories 2 and 3, above). However, since study specimens were randomly selected for heat treatments, we presumably avoided a sex-bias in our study.

Detection and Measurement of Hsp70 (Trials 1 & 3).—Five prepupal head capsules were used per homogenate in each trial to ensure adequate amounts of soluble protein for analyses. (In Trial 3 we used four heads in one sample of *M. apicalis*, but the protein concentration was still sufficient for analysis.) These were homogenized in 10 mM phosphate buffered saline (PBS) at pH 7.6, with 2 mM tosyl arginine methyl ester (TAME) and 0.2% sodium azide. After centrifugation ($16,000 \times g$ for 20 min), the protein content was determined by the Bradford assay (Bradford 1976).

We used one-dimensional SDS-PAGE (Laemmli 1970) to separate 80 μ g of total protein by molecular weight in Trial 1. Homogenate volumes were combined with sample reducing buffer (1:2 ratio) containing bromophenol blue (0.1%). These were denatured for 4 min at 95° C. Proteins were then separated on a minigel (Bio-Rad Laboratories, Hercules, California) at 20V for 8 h. The resulting proteins were then transferred (overnight) to nitrocellulose membranes at 90 mAmps in Tris-glycine-methanol (pH 8.6) buffer using a corresponding miniblott apparatus. Ponceau-S stain was then used to detect general proteins following the transfer (Sambrook et al. 1989). After washing with distilled water and blocking with 3% gelatin TBS, hsp70 family proteins were detected using a monoclonal antibody (1:1000 dilution) for bovine hsp70 (Sigma-Aldrich, St. Louis, Missouri) and an alkaline phosphatase-conjugated secondary antibody (1:3000 dilution).

Two replicate gels were run to confirm the observed banding patterns and to justify further analysis using a modified ELISA test (Yu et al. 1994).

Quantitative measurement of hsp70 using the ELISA test (Trials 1 and 3) involved coating wells of costar microplates (Bio-Rad Laboratories, Hercules, California) overnight with 2000 ng of soluble protein in a buffer of 0.01 M sodium carbonate and bicarbonate buffer (pH 9.6). Plate wells were then washed with PBST (10 mM PBS, 0.05% Tween 20) and then blocked with 1% PBST-BSA for one hour at 37.0° C. Plate wells were then washed (4 × PBST) and alkaline phosphatase-conjugated secondary antibodies were added (1:3000 dilution). After an hour of incubation (37.0° C), plate wells were washed again (6 × PBST) and 200 µl Bio-Rad detection solution was added to each well. After a 60 min incubation at 37.0° C, the optical density 405 nm for each well was recorded with a MR5000 EIA reader (Dynex Technology, Chantilly, Virginia). Each plate contained five bovine hsp70 standards (Sigma-Aldrich, St. Louis, Missouri) that were loaded in triplicate. Each of the three homogenates were also loaded onto the microplates in triplicate, and an average absorbance was calculated for each homogenate. Using the hsp70 standard curve (per microplate), we calculated hsp70 concentrations per homogenate which were used to calculate treatment means.

RESULTS

Trial 1: Tolerance to High Temperatures.—A single hsp70 isoform was detected by one-dimensional SDS-PAGE in Trial 1. We compared hsp70 family protein band intensities between species on nitrocellulose membranes taken from the SDS-PAGE procedure described above for Trial 1. Figure 1 shows a representative sample of these results. In general, except for the highest temperature (55.0° C), bands were detectable for *M. rotundata* at all heat exposures (Fig. 1a). A series of three relatively intense bands appears in the range of 40.0–45.0° C (lanes 3–5) with a decline in intensity thereafter. Overall, bands are fainter for *M. apicalis* with higher intensity around 45.0° C (Fig. 1b). A band was not detectable for this species at 55.0° C.

The ELISA results shown in Fig. 2 are consistent with the banding patterns described above and indicate interspecific differences. First, the two species had significantly different levels of hsp70 as indicated by a two-factor ANOVA ($F = 82.86$, $df = 1$, $P < 0.001$). Mean differences were significant between species for each treatment except the three highest temperature treatments (using Fisher PLSD mean separation tests). Peaks in hsp70 levels are apparent at 42.5° C (5.33 ng/µl) and 45.0° C (5.82 ng/µl) for *M. rotundata* and *M. apicalis*, respectively. A significant decline from the peak for *M. rotundata* first occurs at 47.5° C whereas *M. apicalis* does not decline significantly from its peak until 52.5° C.

Overall survivorship among the nine treatments was significantly different between *M. apicalis* and *M. rotundata* according to a Wilcoxon signed-rank test ($Z = -3.603$, $P < 0.01$). A slight decline in survivorship for both species is first noticeable at 49.0° C (Fig. 3). However, among the *M. apicalis* cells scored as surviving at that temperature, 73.2% (52 of 71) had emerged as adults while only 4.1% (3 of 73) of surviving *M. rotundata* had emerged from their cocoons. Of the brood cells exposed to the 52.5° and 55.0° C treatments, neither *M. rotundata* nor *M. apicalis* emerged from brood cells. However, 43.7% (31 of 71) and 15.9% (11 of 69) of *M. apicalis* larvae were scored as survivors (as evidenced by par-

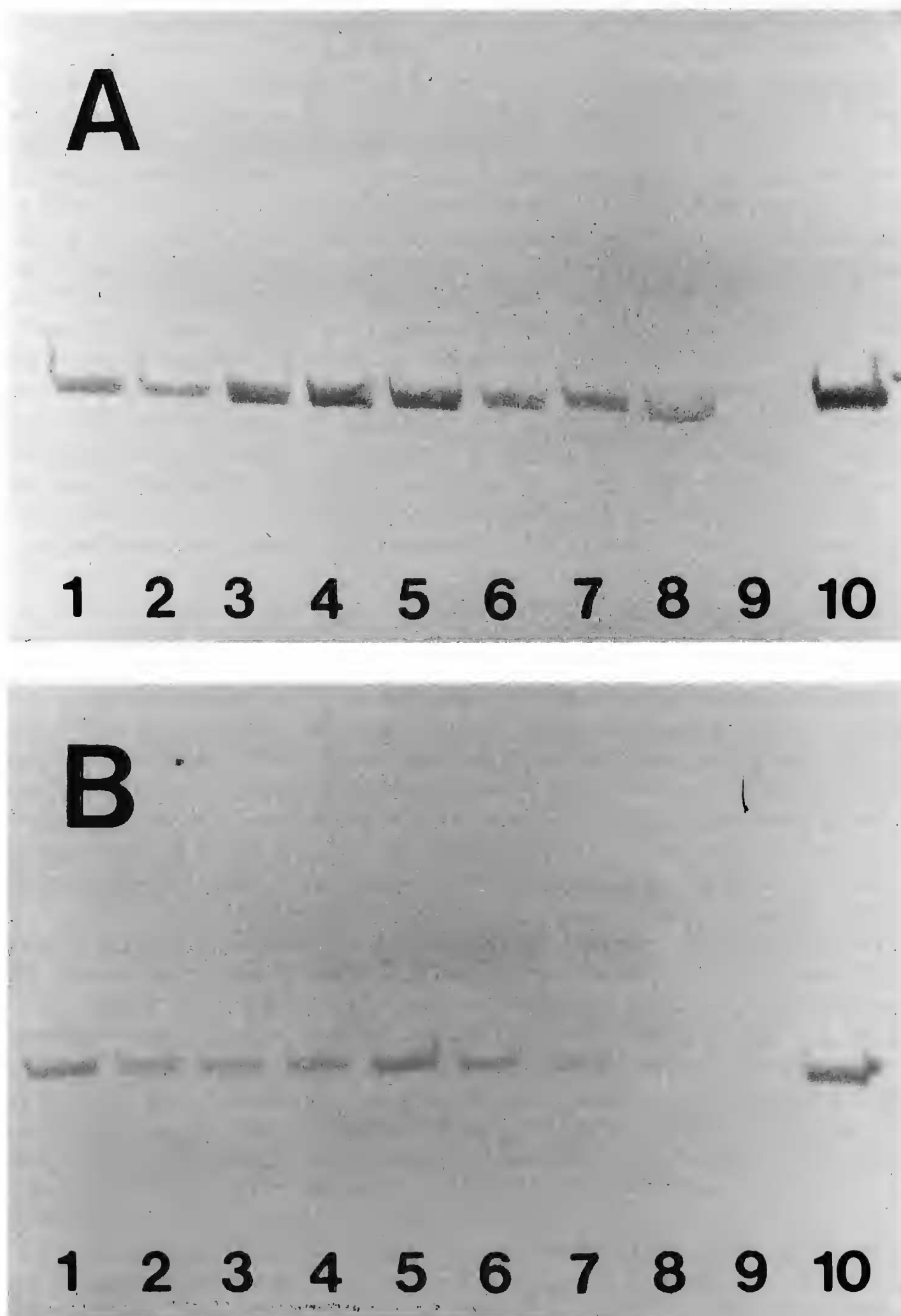


Figure 1. One-dimensional Western blot of hsp70 family proteins as detected with anti-bovine hsp70 antibodies. Each lane contained equal quantities of total protein derived from head capsule homogenates of *M. rotundata* (A) or *M. apicalis* (B). Lanes 1-9 represent ascending temperature treatments (degrees Celsius): 1 = 35.0, 2 = 37.5, 3 = 40.0, 4 = 42.5, 5 = 45.0, 6 = 47.5, 7 = 49.0, 8 = 52.5 and 9 = 55.0. Lane 10 = hsp70 control.

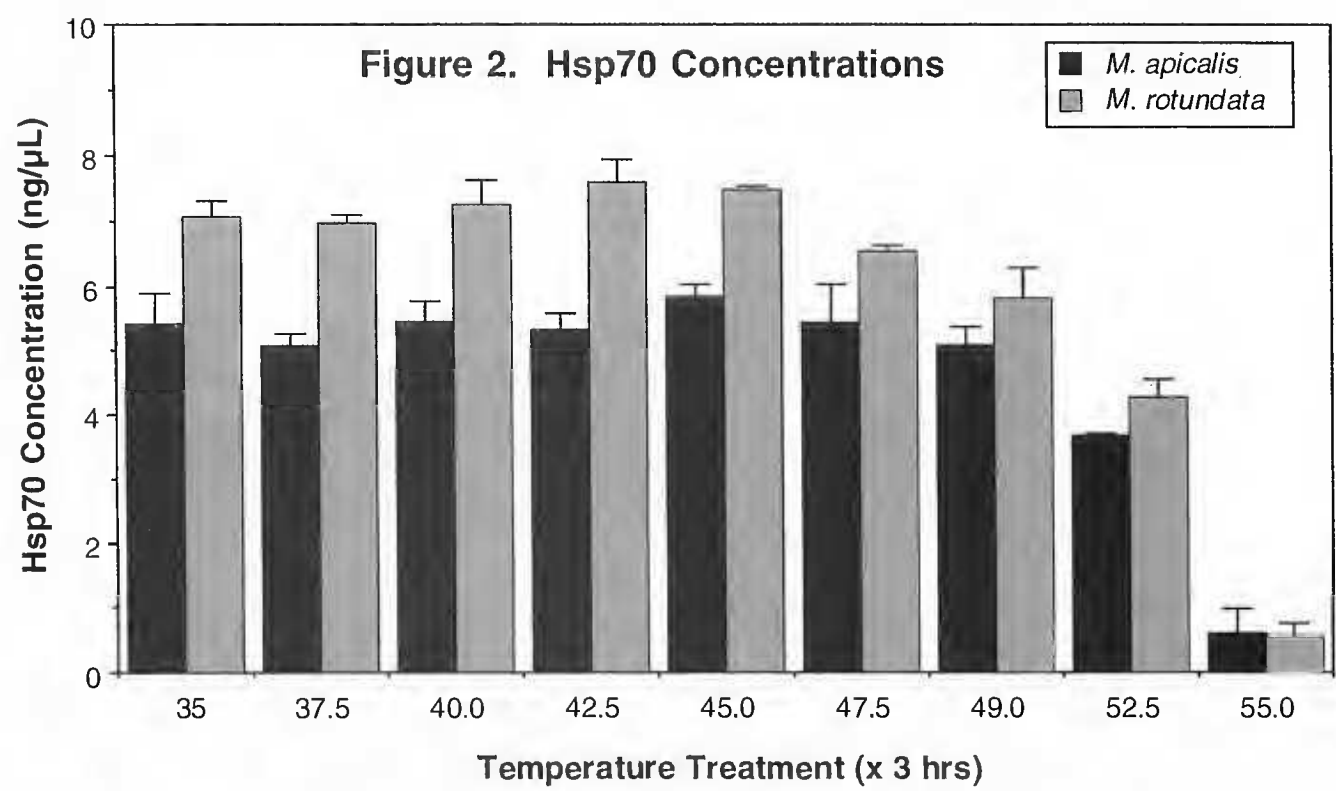


Figure 2. Mean (± 1 SE) concentrations of hsp70 (ng/ μ l) derived from head capsules of *Megachile apicalis* and *M. rotundata* for nine temperature treatments.

asitoid feeding activity) at these two temperatures, respectively. None of these had advanced beyond the prepupal stage. All cocoons showed evidence of parasitoid entry (small circular openings) and no pre-adult bees had survived parasitoid attack after the four-week incubation period. Teneral adults had apparently succumbed to dessication after parasitoid damage to the cocoon.

Trial 2: Survivorship at 50.0° C.—The 50.0° C treatment corroborated our ear-

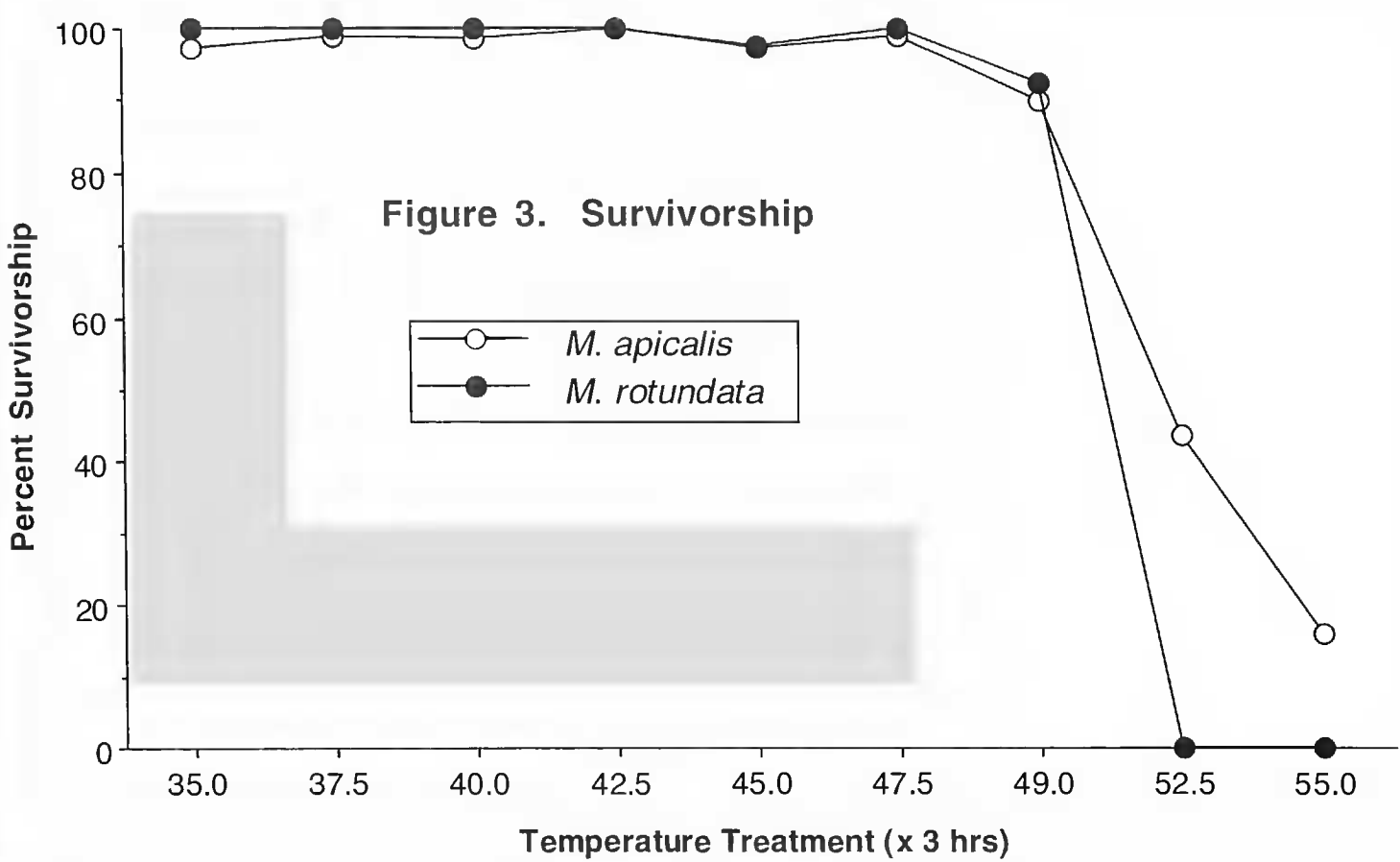


Figure 3. Percent prepupal survivorship of *M. apicalis* and *M. rotundata* compared at nine temperature regimes.

lier result that *M. apicalis* has higher survivorship at the highest temperature treatments in our study. Emergence of adult *Megachile apicalis* from brood cells after six weeks was 40.6% (39 of 96 viable brood cells) relative to 20.0% (18 of 90 viable cells) for *M. rotundata*. Dissection of non-emerged cocoons revealed that 68.8% (66 of 96) of *M. apicalis* brood cells exposed to 50.0° C either successfully emerged, died as teneral adults or advanced to the pupal stage. For *M. rotundata*, 32.2% (29 of 90) of prepupae advanced to the same stages. No parasitoid feeding activity was observed in any cells of either species during this trial. A number of bees that emerged from cocoons showed evidence of teratogenic effects, including at least 28.2% (11 of 39) of *M. apicalis* and 5.6% (1 of 18) of *M. rotundata*. Typically, these malformations related to appendages (e.g., missing or stunted wings and legs).

Trial 3: Stress Proteins in Acclimated vs Heat-shocked Larvae.—Using the same ELISA technique as described above, no significant difference in hsp70 levels was found between *M. apicalis* (5.25 ± 0.749 ng/ μ l) and *M. rotundata* (5.90 ± 1.067 ng/ μ l) when maintained at 25.0° C ($t = -0.503$, $df = 4$, $P = 0.32$). However, a significant difference in levels was recorded after the three-hour exposure to 35.0° C. *Megachile apicalis* contained substantially lower levels of hsp70 than *M. rotundata* (4.15 ± 0.543 vs 7.24 ± 0.481 ; $t = -4.269$, $df = 4$, $P < 0.01$).

DISCUSSION

Although both of our study species appear to tolerate high-temperature environments, the geographic range and nesting habits of *M. apicalis* suggest it has higher thermotolerance than *M. rotundata* (Thorp 1996, Barthell et al. 1998). Our study corroborates these observations with higher survivorship for *M. apicalis* exposed to 50.0° C (and above) while higher stress protein (hsp70) levels were measured in *M. rotundata* in response to elevated temperatures (except at 55.0° C where the most rapid mortality presumably occurred for both species). These differences are likely to be induced by heat-shock since we later failed to find a significant difference between species when samples received a four-day acclimation period at 25.0° C in Trial 3. (Hsp70 levels did, however, differ significantly after the three-hour exposure to 35.0° C.) These results therefore support the conclusion that the elevated levels of hsp70 recorded in *M. rotundata* were a result of temperature-induced stress.

It is unclear whether any species-specific developmental differences influenced our results in the experimental trials. *Megachile apicalis* and *M. rotundata* do have differing flight periods (Thorp et al. 1992) and the developmental sequence from diapausing prepupal to pupal stages just prior to adult emergence could affect hsp70 levels differentially between species. *Megachile rotundata* demonstrates variation among protein concentrations during this transition, for example, and hsp70 appears to be one of those that varies (Rank et al. 1982, 1989; Hranitz & Barthell in press). However, we exposed both study species (together) to at least a three-week cold storage period and we collected cells of *M. apicalis* later in the season (August) to obtain as many of the diapausing generation bees as possible (the same stage as the commercially-harvested *M. rotundata* used in the study).

The literature is replete with evidence that elevated hsp70 levels reflect stress

in organisms (Feder & Hofmann 1999). In insects this pattern has been observed in fruitfly larvae, *Drosophila melanogaster* (Meigen), when exposed to high temperatures in nature (Feder et al. 1997). Cold-shock stress also produces elevated levels of hsp70 in the flesh fly *Sarcophaga crassipalpis* Macquart (Joplin et al. 1990). In diapausing gypsy moths, *Lymantria dispar* L., an elevation in stress proteins occurs in response to both high and low temperatures (Yocum et al. 1991). Both bacterial infection and heat stress increase hsp70 production in honey bees (Severson et al. 1990, Gregorc & Bowen 1999). The significantly higher hsp70 levels we recorded in *M. rotundata* probably reflect lower heat tolerance in this species and is consistent with the northerly distribution of its feral populations in California. However, since the *M. rotundata* specimens used in our study originated from a commercial source in Canada we still await the opportunity to compare individuals of *M. apicalis* and *M. rotundata* sampled from sympatric populations in California.

Although no thermotolerance studies exist in the literature for *M. apicalis*, several such studies exist for the economically important *M. rotundata*. Whitfield and Richards (1992) demonstrated a slightly decreased developmental rate for later instar larvae of *M. rotundata* in the range of 32.0° to 35.0° C. Undurraga and Stephen (1980) found no survivorship of either prepupae or pupae during either brief or extended (as long as 3 h) exposures to 50.0° C, but with high survivorship at 45.0° C. The latter study shows lower survivorship at 50.0° C than in our study for *M. rotundata* (0.0 vs 20.0%). However, these differences could be explained by differing acclimation conditions between studies as well as larval developmental conditions in the original, pre-study environments; the importance of this latter point is emphasized in recent contributions by Kemp and Bosch (2000, 2001). Our criteria for assessing survivorship (based upon parasitoid feeding activity) differed from these other studies as well. Nonetheless, it is clear that *M. rotundata* is severely compromised (> 50.0% mortality) in the prepupal stage when exposed to 50.0° C for a three hour period or less.

Teratogenic effects were not specifically addressed in the mortality studies cited above, nor by Tepedino and Parker (1986), even though we observed them in both species after Trial 2. Such effects were not conspicuous among specimens that emerged from any of the temperature treatments conducted in Trial 1. Although prepupae used in Trial 2 came from the same source as those used in Trial 1 (and were maintained in cold storage), there is a possibility that bees in Trial 2 were somehow physiologically altered between trials. It may also be that other components of our experimental protocol (or these factors in combination with the treatment exposure) produced the effects we observed in Trial 2.

Although no comparative studies measuring survivorship and stress proteins among solitary bee species exist in the literature, such studies have been conducted for marine molluscs. Two blue mussel species, *Mytilus trossulus* Gould and *M. galloprovincialis* Lamarck (collected from northern and southern latitudes, respectively), for example, show significant differences in hsp levels under temperature stress (Hofmann & Somero 1996). *Mytilus trossulus* accumulates higher levels of hsp66 and hsp70 than *M. galloprovincialis* after acclimation to 13.0° C for eight weeks, evidence that the more cold-adapted *M. trossulus* experiences greater physiological stress at temperatures above their normally encountered temperature (ca. 10.0° C). In another study, Tomanek and Somero (1999) studied four

marine snails in the genus *Tegula* collected from sites along the Pacific coast of the U.S.A. One of these, *T. rugosa* (Adams), originated from a more southerly locale than the others (Baja California) and displayed higher onset, peak and inactivation temperatures for hsp70 expression relative to three temperate species collected near Pacific Grove, California. One of the three temperate species, *T. funebris* (Adams), lives in a broader range of habitats (mid- to low-intertidal zones) that can expose it to high air temperatures during low tide. It also has higher onset, peak and inactivation temperatures in comparison with the other two temperate species.

Our findings are consistent with aspects of the marine invertebrate studies cited above. *Megachile rotundata*, a more northerly-distributed species accumulates higher hsp70 levels than *M. apicalis* during heat-treatments in our study. The generally high levels of hsp70 in Trial 1 may have obscured the actual peaks as well as the onset and inactivation temperatures for both species. However, a significant decline from the observed peaks of hsp70 occurred at a lower temperature in *M. rotundata* than in *M. apicalis*, suggesting a higher inactivation temperature for the latter species. The unexpectedly high levels of hsp70 at lower temperatures (relative to acclimated individuals in Trial 3) may reflect a heat-shock response to rapid heating rates ($\geq 10^{\circ}\text{C}$ in < 10 min) used in Trial 1, a condition unlike larvae would experience in nature. Lutterschmidt and Hutchison (1997) caution about such confounding effects in studies of thermotolerance.

Thermotolerance in an invasive species may be an important indicator of the range that it will ultimately occupy in a newly invaded environment. Indeed, thermotolerance has been a key element in predicting the outcomes of other invasive Hymenoptera such as African honey bees (Taylor 1977). Although the original, Eurasian ranges of *M. rotundata* and *M. apicalis* are unclear from reports in the literature, these species are currently occupying ranges in the western USA that are in accord with the thermotolerance patterns recorded in our study. *Megachile apicalis* appears to be able to occupy a higher thermal niche relative to *M. rotundata*, but we still await comparisons with sympatric native species identified in previous studies (Barthell et al. 1998, Frankie et al. 1998). Such comparisons will allow us to determine if thermotolerance is a factor in the rapid range expansion observed for *M. apicalis* since its initial detection in southern California nearly two decades ago (Cooper 1984).

ACKNOWLEDGMENT

We thank D. Nahuliak, R. Bitner and S. Peterson (International Pollination Systems) for their advice and assistance. The management and staff of the Cosumnes River Preserve near Galt, California, facilitated our field collections of *M. apicalis* specimens. In the laboratory, M. Hartless assisted in dissections and preparation of specimens for laboratory analyses. Specimen x-rays were conducted by S. Maslowski (University of California, Davis, Veterinary Medical Hospital). An earlier draft of this manuscript was provided for commentary to G. Frankie (University of California, Berkeley), W. Kemp (USDA, Logan, Utah) and W. Stephen (Oregon State University). Funding for this work was provided by the University of Central Oklahoma's College of Graduate Studies and Research (Deans S. N. Rao and W. R. Radke). The C. P. Alexander Grant (Pacific Coast Entomological Society) provided a partial page charge waiver for this article.

LITERATURE CITED

- Barthell, J. F. 1992. Heterogeneity, invaders, and the population dynamics of some oak forest solitary bee communities. Ph.D. Dissertation, University of California, Berkeley.
- Barthell, J. F., G. W. Frankie & R. W. Thorp. 1998. Invader effects in a community of cavity nesting megachilid bees (Hymenoptera: Megachilidae). *Environ. Entomol.*, 27: 240–247.
- Barthell, J. F., R. W. Thorp, G. W. Frankie, J.-Y. Kim & J. M. Hranitz. 2003 (in press). Impacts of introduced solitary bees on natural and agricultural systems: the case of the leafcutting bee, *Megachile apicalis* Spinola (Hymenoptera: Megachilidae). In Strickler, K. V. & J. H. Cane (eds.). For nonnative crops, whence pollinators of the future? Thomas Say Publications in Entomology: Proceedings, Entomological Society of America, Lanham, Maryland.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248–254.
- Cane, J. H. 2003 (in press). Exotic non-social bees (Hymenoptera: Apoidea) in North America: ecological implications. In Strickler, K. V. & J. H. Cane (eds.). For nonnative crops, whence pollinators of the future? Thomas Say Publications in Entomology: Proceedings, Entomological Society of America, Lanham, Maryland.
- Cooper, K. W. 1984. Discovery of the first resident population of the European bee, *Megachile apicalis*, in the United States (Hymenoptera: Megachilidae). *Entomol. News*, 95: 225–226.
- Feder, M. E., N. Blair & H. Figueras. 1997. Natural thermal stress and heat-shock protein expression in *Drosophila* larvae and pupae. *Funct. Ecol.*, 11: 90–100.
- Feder, M. E. & G. E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Ann. Rev. Physiol.*, 61: 243–282.
- Frankie, G. W., R. W. Thorp, L. E. Newstrom-Lloyd, M. A. Rizzardi, J. F. Barthell, T. L. Griswold, J.-Y. Kim & S. Kappagoda. 1998. Monitoring solitary bees in modified wildland habitats: implications for bee ecology and conservation. *Environ. Entomol.*, 27: 1–12.
- Gregorc, A. & J. D. Bowen. 1999. *In situ* localization of heat shock and histone proteins in honey-bee (*Apis mellifera* L.) larvae infected with *Paenibacillus larvae*. *Cell. Biol. Int.*, 23: 211–218.
- Hofmann, G. E. & G. N. Somero. 1996. Interspecific variation in thermal denaturation of proteins in the congeneric mussels *Mytilus trossulus* and *M. galloprovincialis*: evidence from the heat-shock response and protein ubiquitination. *Mar. Biol.*, 126: 65–75.
- Hranitz, J. M. & J. F. Bartnell. 2003 (in press). Heat shock protein 70 during development of the leafcutting bee, *Megachile rotundata* (Hymenoptera: Megachilidae). *Southwestern Entomol.*
- Joplin, K. H., G. D. Yocum & D. L. Denlinger. 1990. Cold shock elicits expression of heat shock proteins in the flesh fly, *Sarcophaga crassipalpis*. *J. Insect Physiol.*, 36: 825–834.
- Kemp, W. P. & J. Bosch. 2000. Development and emergence of the alfalfa pollinator *Megachile rotundata* (Hymenoptera: Megachilidae). *Ann. Entomol. Soc. Am.*, 93: 904–911.
- Kemp, W. P. & J. Bosch. 2001. Postcocooning temperatures and diapause in the alfalfa pollinator *Megachile rotundata* (Hymenoptera: Megachilidae). *Ann. Entomol. Soc. Am.*, 94: 244–250.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680–685.
- Lutterschmidt, W. I. & V. H. Hutchison. 1997. The critical thermal maximum: history and critique. *Can. J. Zool.*, 75: 1561–1574.
- Peterson, S. S., C. R. Baird & R. M. Bitner. 1992. Current status of the alfalfa leafcutting bee, *Megachile rotundata*, as a pollinator of alfalfa seed. *Bee Sci.*, 2: 135–142.
- Rank, G. H., A. J. Robertson & S. M. Gilmer. 1982. An analysis of major protein species during pupation of *Megachile rotundata*. *Insect Biochem. Mol. Biol.*, 12: 699–705.
- Rank, G. H., A. J. Robertson & J. H. Gerlach. 1989. Crossed immunoelectrophoresis of major protein antigens during pupation of the leafcutting bee *Megachile rotundata*. *Apidologie*, 20: 139–147.
- Sambrook, J., E. F. Fritsch & J. Maniatis. 1989. *Molecular cloning: a laboratory manual* (2nd ed.). Cold Spring Harbor Press, Cold Spring Harbor, New York.
- Severson, D. W., E. H. Erickson, Jr., J. L. Williamson & J. M. Aiken. 1990. Heat stress induced enhancement of heat shock protein gene activity in the honey bee (*Apis mellifera*). *Experientia*, 46: 737–739.
- Stephen, W. P. 1987. *Megachile (Eutricharea) apicalis*, an introduced bee with potential as a domesticable alfalfa pollinator. *J. Kansas Entomol. Soc.*, 60: 583–584.

- Taylor, O. R. 1977. The past and possible future spread of Africanized honeybees in the Americas. *Bee World*, 58: 19–30.
- Tepedino, V. J. & F. D. Parker. 1986. Effect of rearing temperature on mortality, second-generation emergence, and size of adult in *Megachile rotundata* (Hymenoptera: Megachilidae). *J. Econ. Entomol.*, 79: 974–977.
- Thorp, R. W. 1996. Resource overlap among native and introduced bees in California. pp. 143–151. *In* Matheson, A., S. L. Buchmann, C. O'Toole, P. Westrich & I. H. Williams (eds.). *The conservation of bees*. Linnean Society Symposium Series No. 18. Academic Press, London, United Kingdom.
- Thorp, R. W., G. W. Frankie, J. Barthell, D. Gordon, L. Newstrom, T. Griswold, J. Schmidt & S. Thoenes. 1992. Long-term studies to gauge effects of invading bees. *Calif. Agric.*, 46 (1): 20–23.
- Thorp, R. W., A. M. Wenner & J. F. Barthell. 2000. Pollen and nectar resource overlap among bees on Santa Cruz Island. pp. 261–268. *In* Browne, D. R., K. L. Mitchell & H. W. Chaney (eds.). *Fifth California islands symposium, 29 March–1 April 1999*. MBC Applied Environmental Sciences, Costa Mesa, California.
- Tomanek, L. & G. N. Somero. 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: implications for limits of thermotolerance and biogeography. *J. Exp. Biology*, 202: 2925–2936.
- Undurraga, J. M. & W. P. Stephen. 1980. Effect of temperature on development and survival in post-diapausing alfalfa leafcutting bee prepupae and pupae (*Megachile rotundata* (F.): Hymenoptera: Megachilidae). I. High temperatures. *J. Kansas Entomol. Soc.*, 53: 669–676.
- Whitfield, G. H. & K. W. Richards. 1992. Temperature-dependent development and survival of immature stages of the alfalfa leafcutter bee, *Megachile rotundata* (Hymenoptera: Megachilidae). *Apidologie*, 23: 11–23.
- Yocum, G. D., K. H. Joplin & D. L. Denlinger. 1991. Expression of heat shock proteins in response to high and low temperature extremes in diapausing pharate larvae of the gypsy moth, *Lymantria dispar*. *Arch. Insect Biochem. Physiol.*, 18: 239–249.
- Yu, Z., W. E. Magee & J. R. Spotila. 1994. Monoclonal antibody ELISA test indicates that large amounts of constitutive HSP-70 are present in salamanders, turtle and fish. *J. Therm. Biol.*, 19: 41–53.

Received 23 June 2002; Accepted 10 October 2002.